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(71) Applicant: THERAKOS, INC. [US/US]; 437 Creamery Way, Exton, PA 19341 (US).

(72) Inventor: LEE, Kyu, H.; 605 Cornerstone Lane, Bryn Mawr, PA 19010 (US).

(74) Agents: CIAMPORCERO, Audley, A. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933 (US).

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(54) Title: METHOD FOR REMOVAL OF PSORALENS FROM BIOLOGICAL FLUIDS

(57) Abstract

A method for the removal of psoralens and psoralen degradation products is disclosed. The method of the present invention is useful for any biological fluid that has been treated with psoralens, including blood and blood fractions and components derived therefrom. Biological fluids treated according to the method of the present invention are substantially free from any residual psoralens or psoralen degradation products.

# TITLE OF THE INVENTION

METHOD FOR REMOVAL OF PSORALENS FROM BIOLOGICAL FLUIDS

## BACKGROUND OF THE INVENTION

Recently, because of potential risks involved with donated blood, methods for inactivating pathogenic agents that may be found in donor blood or blood components are being actively investigated. One of the most promising approaches is inactivating pathogenic agents by photochemical treatment. One of the main problems in most photochemical treatment methods is reducing the residual photosensitizer or its decomposed products in the treated blood to sufficiently low level so that the treated blood or blood product can be transfused to patient. Even though all donor blood is tested for possible contamination with known pathogens it is currently not possible to completely eliminate all contaminated blood from the donor blood pool.

This is caused by several circumstances. For instance, when a person is infected with viruses such as human immunodeficiency viruses (HIV) which causes AIDS, there is a period during which the anti-HIV antibody titer is too low for positive detection by current screening tests. Therefore, blood donated by an HIV infected person during this period may pass the antibody screening tests and could infect any recipients of the donated blood or blood products made therefrom. Also, there is always the possibility that the donated blood is contaminated by unknown or undetected pathogens. For these reasons currently there is an urgent need for methods to eliminate those undetected pathogens in the donated blood or blood components derived therefrom for human use.

Wiesehahn et al. (U. S. Patent No. 4,727,027; 4748,120; and 5,176,921) and Isaacs et al. (U.S. Patent No. 5,139,940) described methods for deactivating pathogens in biological fluids by UVA irradiation in the presence of psoralen derivatives such as 8-methoxy psoralen(8-MOP), 4'hydroxymethyl-4, 5',8-trimethylpsoralen (HMT); 4'-aminomethyl-4', 5'8-trimethylpsoralen(AMT), or other psoralen derivatives. In this process only a small fraction of the total amount of psoralen compound added is consumed in inactivating those pathogens and the remainder of the added psoralen compound either remains in the treated blood as original psoralen compound or remains in the treated blood as psoralen decomposition products.

The amount of these residual compounds in the treated blood or blood component could be very substantial and when a patient is transfused with this treated blood or blood component the patient may be exposed to psoralens or psoralen degradation

products. This exposure to psoralens or psoralen degradation products may in turn cause undesirable effects on the patient such as phototoxicity or other toxic effects associated with psoralen and their decomposition products. Therefore, it is highly desirable to remove the remaining psoralen derivatives or decomposed psoralen products from the treated blood or blood component before any human use.

Currently there are no methods published which have been shown to remove the psoralen compounds and their decomposition products from blood and blood products.

## Summary of the Invention

The present invention is drawn to a method for the removal of psoralen compounds and their decomposition products from psoralen-treated biological fluids, including but not limited to, blood and blood products. The method of the present invention utilizes a psoralen-adsorbent material which is contacted with the psoralen-treated biological fluid, such as blood or blood products. Biological fluids, blood or blood products that contain psoralen compounds or their decomposition products are treated according to the method of the present invention to produce a biological fluid, blood and blood components that are substantially free from psoralen compounds or psoralen decomposition products.

## Brief Description of the Drawings

Figure 1 shows an overall general view of the usage of this adsorption device. The first container(9) contains already irradiated blood or blood component(11) which contains residual psoralen or psoralen derivatives such as 8-MOP, AMT, HMT or other psoralen derivative and its decomposition products during earlier ultraviolet A irradiation. The treated fluid(11) is pumped by the pump(13) through the adsorption device(1), where the residual photosensitizer(s) or its byproducts are removed, into the second container(10).

Figure 2 shows a vertical cross-sectional view of the adsorption device(1). The cartridge is made of inlet cap(4), outlet cap(5), body casing(6), two stainless steel screens(7), and adsorbent(8). The stainless screens(7) contain the resin beads inside the cartridge and prevent them from coming out of the cartridge.

Figure 3 shows a horizontal cross-sectional view of the device(1). The adsorbent(8) is microporous beads of the size approximately 0.1-2mm in diameter and made from polystyrene or polystyrene copolymerized with divinylbenzene. These microporous beads have pore sizes in the range of molecular level, 10-1000 Angstroms, and

large pore surface area, 100-1,000 square meter per gram of the adsorbent. Good examples are XAD-4 and XAD-16 resin beads made by Rohm and Haas Company.

Figure 4 shows a cross-sectional view of another design of this invention. Here the adsorbent(14) is made of microporous fibers(14) instead of beads. The fibers could be in woven or non-woven configuration. By using fibers instead of beads the stainless steel screens(7) can be eliminated.

Figure 5 shows a cross-sectional view of the same device shown in figure 4. Here the cross-sections of the adsorbent fibers are shown. These adsorbent fibers are woven with other fine threads.

Detailed Description of the Invention

It is the purpose of this invention to develop a method to remove the residual photosensitizers such as psoralen or its derivative(s) and its decomposition products, if any, from biological fluids such as treated blood or blood components so that the treated biological fluids can be transfused into patients substantially free from residual photosensitizer(s). Biological fluids that are suitable for use in the method of the present invention include, but are not limited to, whole blood, serum, plasma, blood fractions such as platelets, red cells, and buffy coat, extracts of blood or blood fractions such as proteins purified therefrom, and any biological fluid that has been treated with one or more psoralen compound.

Many psoralen adsorbent materials are suitable for use in the method of the present invention, and different physical forms of these materials can be made and are suitable for use in the method of the present invention. For instance, activated carbon in the form of microporous beads or fibers is a good psoralen adsorbent. But it has been found that activated carbon may also adsorb other components from blood or blood products. Therefore, its application in the method of the present invention is suitable only if the activated charcoal does not also remove a desirable component of the treated biological fluid. The preferred adsorbent materials for use in the method of the present invention are ones which adsorb the psoralens and psoralen decomposition products with minimum adsorption capacity for other desired components such as the components of blood and blood products for human use.

Microporous polymeric beads such as those made from polystyrene and polystyrene copolymerized with divinylbenzene are the preferred adsorbent materials for use in

the method of the present invention for psoralen, psoralen derivatives and their photodecomposition products.

It is readily apparent to those of ordinary skill in the art that virtually any fluid is suitable for use in the method of the present invention. In particular, any biological fluids that have been treated with psoralen compounds are suitable for use in this method of the present invention. Biological fluids that are commonly exposed to psoralen compounds include, but are not limited to, whole blood, plasma, serum, and any components isolated from blood or blood fractions. Psoralen compounds have been used for a variety of purposes which include the sterilization of human blood and blood-derived products to prevent transmission of hepatitis viruses, herpes viruses, HIV and any other infectious or oncogenic entity derived from blood donors; the sterilization of cell culture-derived biologicals, such as interferons, enzymes, hormones and vaccines, to inactivate any viral or nucleic acid contaminants; and therapeutically in humans by treating patients with psoralens, and then irradiating the blood in an extracorporeal circuit, followed by the return of the psoralen-treated blood to the patient.

It is also readily apparent to one of ordinary skill in the art that a variety of different psoralen-adsorbent materials are suitable for use in the method of the present invention. Examples of suitable types of psoralen-adsorbent materials include, but are not limited to, activated carbon beads or fibers which are uncoated or coated with biocompatable materials, ion exchange resins such as Dowex beads (commercially available from Dow Chemical Company, Midland Michigan), and amberlite beads (commercially available from Rohm and Haas Company, Philadelphia, Pennsylvania), with polystyrene and polystyrene copolymerized with divinylbenzene being most preferred.

It is readily apparent to those skilled in the art that the psoralen-treated biological fluid is contacted with the psoralen-adsorbent material in a variety of ways. For example, the biological fluid may be mixed in a batchwise fashion with the psoralen-adsorbent material, followed by removal of the psoralen-adsorbent material by standard separation means such as filtration or gravitational separation. Alternatively the psoralen-adsorbent material may be placed inside a standard chromatographic device such as a column through which is passed the psoralen-containing biological fluid.

It is also readily apparent to those skilled in the art that virtually any psoralen compound that is suitable for use in biological fluids, is suitable for use with the

method of the present invention. Psoralen compounds are well known in the art and are described in U.S. Patent 4,321,919; and U.S. Patent No. 4,960,408. Commonly used psoralen compounds include, but are not limited to, psoralen; 8-methoxy-psoralen; 4,5'8-trimethylpsoralen; 5-methoxypsoralen; 4-5'dimethyl-psoralen; 4,8-methoxypsoralen; 4-methylpsoralen; 4,4-dimethylpsoralen; 4'-hydroxymethyl-4,5',8-trimethylpsoralen; and 4'-aminomethyl-4,5',8-trimethylpsoralen.

The following Examples are provided to illustrate the present invention without, however, limiting the same thereto.

Example 1

In this experiment to demonstrate the adsorption capacity of styrene or styrene copolymer beads for psoralen derivatives, a glass pipette was used as a resin container and glass wool was used in place of stainless steel screen to keep the beads inside the pipette. A total of 8 grams of XAD-4 resin beads (commercially available from Rohm and Haas Co.) was filled into a pipette. Balls of glass wool were put at the bottom and top of the resin bed inside the pipette. The total bed volume of the resin beads was 11.4 mL. Several gallons of 0.5 ug/mL AMT (psoralen) solution in water was made, pumped through this small XAD-4 resin column, and AMT concentrations in the effluent was measured over time. The results are shown in Table 1.

Table 1
AMT Adsorption on XAD-4 Resin Column

| Run No. | Perfusion Rate mL/min. | Percent Leakage In Last Sample | Total Volume Treated - mL |
|---|---|---|---|
| 1 | 19.5 | 2.4 | 800 |
| 2 | 5.7 | 0.0 | 1,370 |
| 3 | 10.2 | 0.0 | 1,230 |
| 4 | 21.1 | 7.4 | 1,254 |
| 5 | 35.5 | 0.0 | 1,414 |
| 6 | 50.1 | 4.1 | 980 |
| | | | total 7,048 |

The test was carried out at six different flow rates with the same cartridge. As the flow rate increases the resident time of the perfusate in the resin cartridge decreases allowing less time for adsorption to take place. Therefore, if the adsorption rate is slow or the capacity is low, the AMT concentration in the effluent should increase. The test results show that the AMT concentration in the effluent is practically zero and not effected by flow rate increase. These results show that XAD-4 resin beads have extremely high affinity for AMT both in capacity and adsorption rate.

What is claimed is:

1. A method for the removal of psoralen compounds and psoralen degradation products from biological fluids, comprising:

a) contacting a biological fluid containing psoralen or psoralen degradation products with a psoralen-adsorbent material; and

b) separating and collecting the biological fluid from the psoralen-adsorbent material to provide a biological fluid substantially free from psoralen or psoralen degradation products.

2. The method of claim 1 wherein the biological fluid is serum.

3. The method of claim 1 wherein the biological fluid is plasma.

4. The method of claim 1 wherein the biological fluid is red blood cells.

5. The method of claim 1 wherein the biological fluid is whole blood.

6. The method of claim 1 wherein the psoralen-adsorbent material is selected from the group consisting of polystyrene and polystyrene divinylbenzene copolymer.

7. The method of claim 1 wherein the psoralen is 8-methoxypsoralen.

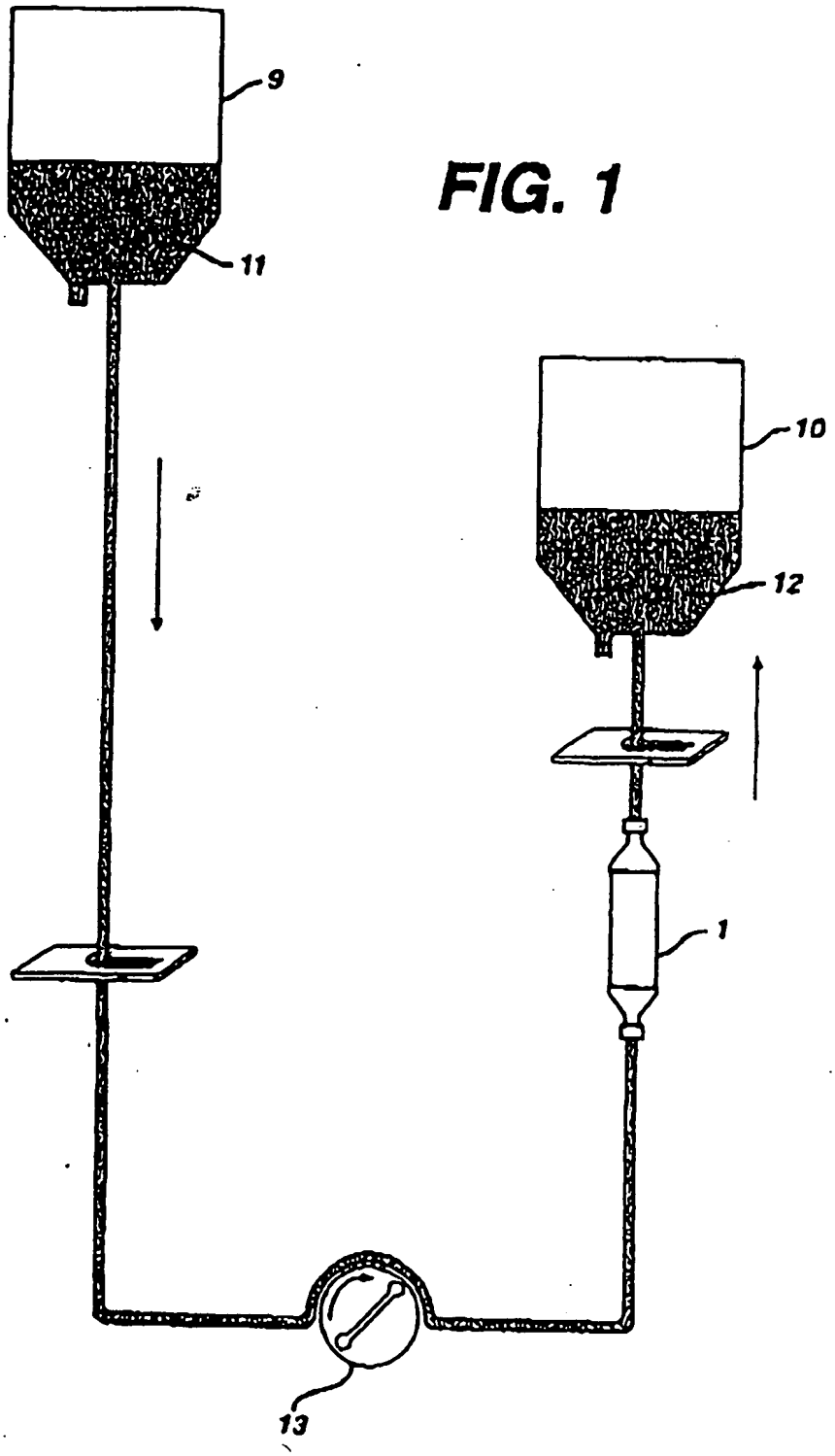



FIG. 1

FIG. 2

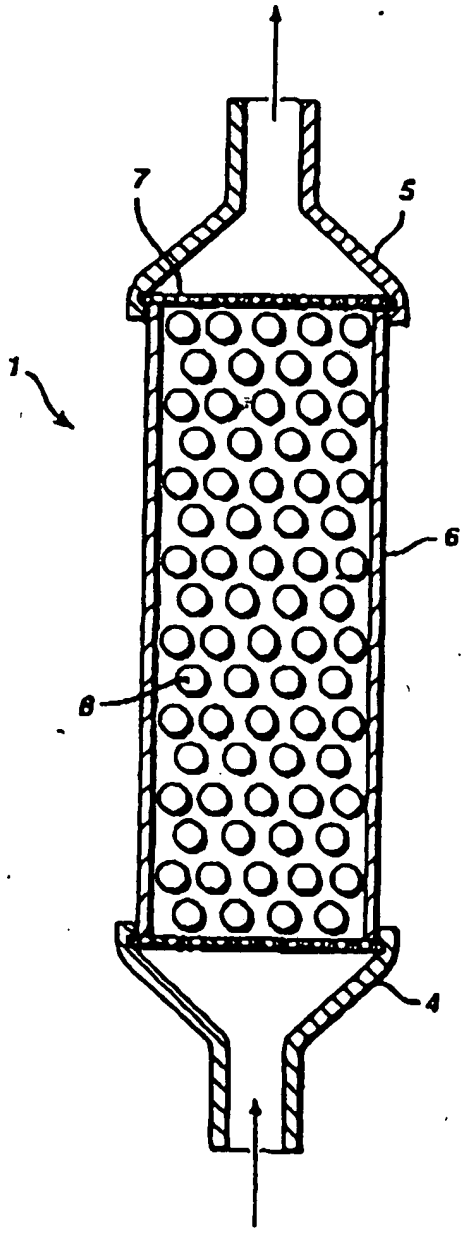



FIG. 3

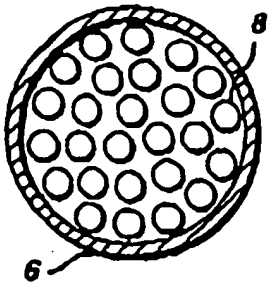


FIG. 4

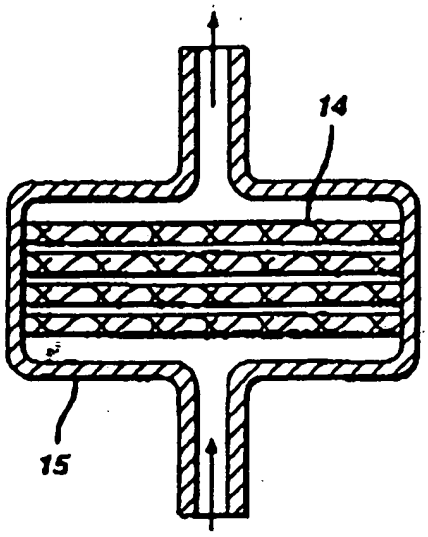
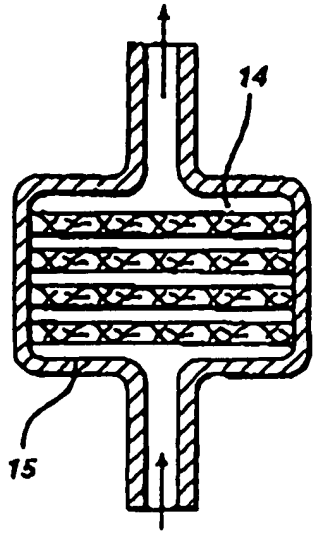



FIG. 5



# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05785

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC(6) :A01N 1/00
US CL : 435/2, 238, 269, 272;530/380
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/2, 238, 269, 272; 530/380

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS; CAPLUS; BIOSIS; EMBASE; MEDLINE; JICST-E; WPIDS
SEARCH TERMS: PSORALENS, STYRENE, POLYSTYRENE, DIVINYLBENZENE,

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No |
|---|---|---|
| X | MARGOLIS-NUNNO, H. et al. Elimination of potential mutagenicity in platelet concentrates that are virally inactivated with psoralens and ultraviolet A light. Transfusion. 1995, Volume 35, pages 855-862, especially page 855 and 857-858 and 860. | 1-7 |
| Y | US 4,693,981 A (WIESEHAHN et al.) 15 September 1987, col. 6, lines 55-70. | 1-7 |
| Y | US 4,748,120 A (WIESEHAHN) 31 May 1988, col. 1, line 25-35. | 1-7 |
| Y | Database WPIDS on STN, Derwent Information Ltd., AN 88-023972, JP 62283198 A (HASEGAWA CO. LTD.) 09 December 1987, abstract. | 1-7 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 26 JUNE 1997 | 12 AUG 1997 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No.: (703) 305-3230 | Authorized officer<br>CHHAYA SAYALA<br>Telephone No. (703) 308-0196 |

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